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13. ABSTRACT (Maximum 200 words)

The objective of this research project was to study the mechanisms by which non-genotoxic or epigenetic chemicals induce multiple disease endpoints such as birth defects, tumor promotion, reproductive- and neurotoxicities. The purpose is to develop a "biologically-based" risk assessment model for human exposure to this class of toxic chemicals. The working hypothesis to have been tested was non-genotoxic chemicals disrupted homeostatic control of cell proliferation, differentiation and adaptive responses of differentiated cells. Three specific aims were designed to be tested (e.g., test a series of toxicants of interest to the USAFOSR for their ability to inhibit gap junctional function; to examine if these toxic chemicals alter the redox state of the cells; to determine if these chemicals alter apoptosis frequency via some oxidative damage-induced signal transduction mechanism). Results showed a structure-function relationship between PAH molecules and inhibition of gap junctions ; jet fuels JP8 and JP4 were inhibitory to gap junctions; and perfluorinated fatty acids with chain length of 7 to 10 carbons were inhibitory to gap junctions.

14. SUBJECT TERMS Gap junctions, oxidative stress, epigenetic toxicology, non-genotoxic, tumor promoters, neurotoxicants, chemical toxicology, reproductive toxicants

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FINAL TECHNICAL REPORT

"OXIDATIVE STRESS, SIGNAL TRANSDUCTION, CELL-CELL COMMUNICATION"

F49620-95-1-0303

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OBJECTIVES:

The basic hypothesis to be tested is that chemicals which produce oxidative damage in cells can trigger various signal transducting mechanisms, which, in turn, can inhibit gap junctional intercellular communication (GJIC) at the transcriptional, translational or posttranslational levels and inhibit apoptosis or programmed cell death.

Four specific aims were proposed to test this hypothesis:

Aim 1: To determine if a series of USAFOSR-toxicants can inhibit GJIC, using a series of human and rodent (for species comparison studies) cells in vitro, using the scrape-loading/dye transfer technique.

Aim 2: To examine the potential mechanism by which an inhibitor of GJIC may have altered the redox state and altered the function of gap junctions.

Aim 3: To examine if any of the chemicals which inhibit GJIC via some oxidative damage also inhibits apoptosis in the same cells.

Aim 4: If time permits, we will attempt to remediate the toxic chemical or to prevent the toxicity (inhibition of GJIC) by some chemopreventive strategy.

Because of notification early into the start of this project that it would not be continued for the remaining two years, and because we were instructed to focus on aim one, we shifted the emphasis from our most promising aim 2 to aim 1. We were, however, able to accomplish work on aim 2 as well as aim 1.

STATUS OF EFFORT:

Jet fuels, JP8 and JP4, naphthalene, anthracene, quadricyclane, perfluorooctanoate (PFOA), and perfluordecanoate (PFDA), are all chemicals of interest to the USAF and have been assessed for an effect on gap junction intercellular communication (GJIC). The down regulation of GJIC has been implicated in the induction of mitogenesis and tumor promotion. The results are as follows: (1) Quadricyclane was very cytotoxic and did not inhibit GJIC; (2) Jet fuels JP8 and JP4 were both inhibitory to GJIC at high but non-cytotoxic doses (5 ul fuel ml of cell culture medium); (3) Naphthalene was inhibitory to GJIC but anthracene was not. A structure function relationship between the methylated isomers of anthracenes was determined in which methylation at the top of the benzene ring which formed an angular pocket inhibited GJIC, whereas methylation that does not form a pocket was not inhibitory to GJIC; (4) Perfluorinated fatty acids with a chain length of 7 to

10 carbons, which included PFOA and PFDA, were inhibitory to GJIC, but chain lengths of 2 to 5 carbons and 16 to 18 carbons were not inhibitory to GJIC.

Many chemicals are known to also induce oxidative stress, such as the peroxisome proliferators PFOA and PFDA. We, therefore, determined if oxidants such as H_2O_2 can also inhibit GJIC. H_2O_2 was shown to reversibly inhibit GJIC at a non-cytotoxic dose in glutathione (GSH) sufficient but not deficient cells. This is a very significant discovery demonstrating that the antioxidant GSH, is actually needed for the signal transduction mechanism of H_2O_2-linked down-regulation of GJIC.

In summary, our results show distinct relationships between the structures of chemicals and their effects on GJIC activity which can play an important role in the predictive assessment of the epigenetic toxicity of chemicals. In addition, we have advanced our knowledge that GSH, a key intracellular antioxidant, also plays a role in signal transduction relative to GJIC.

ACCOMPLISHMENTS/NEW FINDINGS:

These recent findings have (a) demonstrated, again, that non-genotoxic chemicals can inhibit gap junctional intercellular communication (GJIC) at non-cytotoxic doses, in a dose dependent and reversal fashion with "thresh-hold" characteristics; (b) these toxic chemicals, by inducing oxidative stress, trigger signal transduction mechanisms which down regulate GJIC; and (c) by inhibiting GJIC, they can inhibit apoptosis or programmed cell death, stimulate the proliferation of initiated cells to act as tumor promoters, and block differentiation of normal cells to cause birth defects or block normal functions of differentiated cells to cause neurotoxicities.

These finds also are on the cutting edge of the field of "epigenetic toxicology" in that they are not only demonstrating the value of using GJIC as a "biomarker" for the practical use of screening and identifying potentially toxic chemicals that the US Air Force needs to know about, but also these findings are of use to the basic understanding of the biochemical mechanisms by which these chemicals work. This knowledge will give insights to preventive measures to take when exposed to such chemicals and to the modeling of realistic biological risk assessment models. It should be obvious that this approach to the characterization of the mechanisms of toxicity of non-mutagenic chemicals is directly relevant to the AF mission.

PERSONNEL SUPPORTED:

James E. Trosko, Ph.D. (Principle Invest.) [15%-12 months]
Brad Upham, Ph.D. (Research Associate) [100 %-12 months]
Nestor DeoCampo (Graduate Student) [100 %-12 months]
Heather de Feijter-Rupp (Technician) [100%-12 months]

PUBLICATIONS:

1. Upham, B.L., K.-S. Kang, H.-Y. Cho, and J.E. Trosko (1996). Hydrogen peroxide inhibits gap junctional communication in glutathione sufficient but not deficient cells. *Carcinogenesis* (accepted pending revisions).

2. Upham, B.L., L.M. Weis, A.M. Rummel, S.J. Masten, and J.E. Trosko (1996). The effects of anthracene and methylated anthracenes on gap junctional intercellular communication in rat liver epithelial cells. *Fund. Appl. Toxicol.* **34**, 260-264.

3. Upham, B.L., N.D. DeoCampo, and J.E. Trosko (1996). The inhibition of gap junctional intercellular communication by perfluorinated fatty acid with specific chain lengths of 7 to 10 carbons (in preparation).

INTERACTIONS/TRANSITIONS:

A. Participation/Presentation at Meetings, Conferences, Seminars:

1. DeoCampo, N.D., B.L. Upham, and J.E. Trosko (1996). The role of gap junctional communication in the toxicity of quadricyclane, perfluorodecanoic acid, perfluorooctanoic acid and jet fuels JP8 and JP4. *Fund. Appl. Toxicol.* (suppl.) 30, 1065. (Also presented at the 1996 Regional Cancer Society Annual meeting.)

2. Upham, B.L., L.M. Weis, A.M. Rummel, S.J. Masten, and J.E. Trosko (1996). The biological effects of methylated anthracenes on gap junctional intercellular communication depends on the ring position of the methyl group. *Fund. Appl. Toxicol.* (suppl.) 30, 1066. (Also presented at the 1996 Regional Cancer Society Annual meeting.)

3. Weis, L.M., B.L. Upham, A.M. Rummel, S.J. Masten, and J.E. Trosko (1996). Determining a correlation between structural elements of polycyclic aromatic hydrocarbons and inhibition of gap junctional intercellular communication. Center of Microbial Biology and Hazardous Waste Research Center Annual Conference, Lansing, Michigan.

4. Upham, B.L., K.-S. Kang, H.-Y. Cho and J.E. Trosko (1996). Hydrogen peroxide inhibits gap junctional intercellular communication in glutathione sufficient but not glutathione deficient cells. Center of Microbial Biology and Hazardous Waste Research Center Annual Conference, Lansing, Michigan.

5. Upham, B.L., K.-S., H.-Y. Cho, and J.E. Trosko (1996). Hydrogen peroxide inhibits gap junctional intercellular communication via hyperphosphorylation of connexin43 in glutathione sufficient but not deficient cells. Keystone symposia on oxidative stress.

6. Upham, B.L., "The effect of perfluorinated fatty acids on gap junctional intercellular communication." Seminar presented to 3M, St. Paul, Minnesota, June 4, 1996.

7. Invited lecturer, "The role of radiation in the multistage carcinogenic process". National Institute of Radiological Sciences, Chiba, Japan, Jan. 29, 1996 (J.E. Trosko).

8. Invited lecturer, "Role of tumor promoters, oncogenes, and tumor suppressor genes on the modulation of gap junctional intercellular communication: An integrative theory of carcinogenesis". Kyoto Prefectural Medical School, Kyoto, Japan, Jan. 30, 1996 (J.E. Trosko).

9. Invited lecturer, "Modulation of cell-cell communication by carcinogens, oncogenes, tumor suppressor genes as an integrative theory of carcinogenesis". Kyoto University, Dept. of Radiation Genetics, Jan. 31, 1996 (J.E. Trosko).

10. Invited lecturer, "Oxidative stress, signal transduction and cell-cell communication". Central Institute of Electric Power Research, Tokyo, Japan, Feb. 1, 1996 (J.E. Trosko).

11. Invited lecturer, "The role of oxidative stress and signal transduction in the modulation of cell-cell communication during carcinogenesis". National Institute of Environmental Health Sciences, Tokyo, Feb. 2, 1996 (J.E. Trosko).

12. Invited lecturer, "The discovery of the 'Biological Rosetta Stone': The role of cell-cell communication in carcinogenesis". Seoul National University, College of Veterinary Medicine, Seoul, Korea, Feb. 12, 1996 (J.E. Trosko).

13. Invited lecturer, "The use of cell-cell communication as a biomarker for chemical safety and toxicity evaluations". Korea Institute of Advanced Science and Technology, Taejon, Korea, Feb. 13, 1996 (J.E. Trosko).

14. Invited lecturer, "The use of cell-cell communication as an endpoint for drug development and drug safety evaluation". National Institute for Safety Research, Seoul, Korea, Feb. 15, 1996 (J.E. Trosko).

15. Invited lecturer, "The role of chemical, oncogene and tumor suppressor gene modulation of cell-cell communication: An integrative theory of carcinogenesis". Seoul National University School of Medicine, Feb. 15, 1996 (J.E. Trosko).

16. Invited symposium speaker, "Oxidative stress, signal transduction and intercellular communication in carcinogenesis". Biological Effects of Radiation Injury Symposium, Minsk, Belarus, March 22, 1996 (J.E. Trosko).

17. Invited lecturer, "Use of laser imaging to study mechanisms of carcinogenesis", Pathology 891, MSU, April 8, 1996 (J.E. Trosko).

B. Consultant and Advisory Functions to other Laboratories:

1. Consulted for 3M, St. Paul, Minnesota 55144-1000 on June 4, 1996, concerning the use of gap junctional communication assays in determining the epigenetic toxicity of fluorinated compounds. Primary contact was Robert D. Howell, Senior Environmental Chemist and International Environmental Contact (B.L. Upham).

2. Consulted with Dr. Tohru Inoue of the Natl. Institute of Health Sciences in Tokyo and Dr. Kouichi Tatsumi of the Natl. Institute of Radiological Sciences (Chiba, Japan) and started collaborative projects related to chemical/radiation-induced oxidative stress (J.E. Trosko).

3. Dr. Frank Witzmann, University of Indiana/Purdue at Columbus, Indiana. To collaborate on a project to use 2-D gel electrophoresis in order to compare toxicant-altered proteins in liver cells *in vivo* to those of rat liver epithelial cells *in vitro*. Two experiments were performed which showed that the whole liver preparation had many proteins shared by the pure cultures of rat liver epithelial cells (the presumptive target cells for carcinogenesis). However, the whole liver had proteins of multiple cell types, must not

contributing to the cells which give rise to toxicant induced liver cancer. These preliminary experiments had great promise for comparative subtractive analyses, however, because of the notice of termination of this project, the collaboration terminated (J.E. Trosko).

3. Dr. Adriana Haimovitz-Friedman, Dept. of Radiation Oncology, Sloan-Kettering Cancer Center, New York. To collaborate on a project to identify the sphingomyelin pathway that exists in rat liver oval cells which affect ceramide induced apoptosis. This project just got started. This project's aim is to determine why ceramide can induce apoptosis in some cell types, while it can induce differentiation or proliferation in other cell types (J.E. Trosko).

C. Transitions:

The fundamental application of our findings is that the scientific field, in general, and the regulatory agencies (e.g., new EPA Cancer Guidelines) have acknowledged the role of GJIC in the tumor promotion phase of carcinogenesis. More and more chemical and drug companies (e.g., Sumitomo Chemical Corp., and Nippon Shinyaku Pharmaceutical Company of Japan) and government institutions (National Institute of Health Sciences of Japan and the U.S. EPA) have incorporated the concept of modulated GJIC as a marker for risk identification and assessment. Even 3M Company is seriously examining the possibility of utilizing GJIC into their toxicology program.

NEW DISCOVERIES, INVENTIONS, PATENT DISCLOSURES:

None.

HONORS/AWARDS:

Dr. Trosko received the Kenneth P. DuBois Award from the Midwest Society of Toxicology Chapter (1995) and a Japan Science and Technology Award for a six-week consultantship to visit the National Institute of Radiological Sciences in Chiba and the National Institute of Health Sciences in Tokyo (Jan.-Feb., 1996)

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May 6, 1996

Dr. James E. Trosko
Department of Pediatrics & Human Development
Michigan State University , B240 Life Sciences
East Lansing, Michigan 48824

Re: Manuscript S054

Dear Dr. Trosko:

Thank you for the opportunity to consider your manuscript "The effects of anthracene and methylated anthracenes on gap junctional intracellular communications in rat liver epithelial cells" for publication in *Fundamental and Applied Toxicology.* I am pleased to report that your manuscript has been reviewed by two members of our Editorial Board and judged acceptable for publication pending revision. The reviewers have offered a number of suggestions which I think you will find helpful in revising your manuscript for publication. I support the reviewers' comments and ask that you consider them carefully. On return of your revised manuscript, please provide a letter describing your responses to the reviewer's comments and resulting changes in the manuscript, if any. If you do not feel a suggested change is warranted, please provide a written rebuttal.

I would appreciate it if you could send your revised manuscript to me within 60 days of the date of this letter. Due to processing deadlines, we must consider manuscripts not returned within 60 days as being withdrawn. We will need an original of the revised manuscript plus two copies--one of which should have all changes marked with highlighter.

If you have any questions or find that you need more time to complete your revisions, please contact me at 919-541-3252.

Sincerely,

HB Matthews

H. B. Matthews, Ph.D.
Associate Editor

Enclosure

Extension to Aug 6